

What is claimed is:

1. A composition comprising two or more isolated nucleic acids selected from the group consisting of an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity.

2. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.

3. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus,

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wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle, and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

4. A method of making the population of alphavirus replicon particles of claim 2 comprising:
    - A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
      - (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
      - (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
      - (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;
- and with at least one of said helper RNAs lacking an alphavirus packaging signal;
- wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is

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unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on

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permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and

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D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 2.

5. The method of claim 4, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises nucleic acid sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

6. A method of making the population of alphavirus replicon particles of claim 3, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

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wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particle, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

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wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof is modified to inhibit reverse transcriptase activity, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper

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RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 3.

7. The method of claim 6, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises nucleic acid sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

8. The method of claim 6, wherein only at least one of the first population of alphavirus particles, the second population of alphavirus particles and the third population of alphavirus particles comprises particles wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations.

9. A population of alphavirus replicon particles produced by the method of claim 4.

10. A population of alphavirus replicon particles produced by the method of claim

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6.

11. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 1 in a pharmaceutically acceptable carrier.

12. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 2 in a pharmaceutically acceptable carrier.

13. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 3 in a pharmaceutically acceptable carrier.

14. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 9 in a pharmaceutically acceptable carrier.

15. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 10 in a pharmaceutically acceptable carrier.

16. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 1 in a pharmaceutically acceptable carrier.

17. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 2 in a pharmaceutically acceptable carrier.

18. A method of treating or preventing infection by human immunodeficiency

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virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 3 in a pharmaceutically acceptable carrier.

19. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 9 in a pharmaceutically acceptable carrier.

20. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 10 in a pharmaceutically acceptable carrier.

21. A composition comprising two or more isolated nucleic acids selected from the group consisting of an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof.

22. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment

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thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle.

23. A composition comprising a population of alphavirus replicon particles comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon particle, and further wherein the alphavirus replicon particles comprise a replicon RNA or at least one structural protein which comprises one or more attenuating mutations.

24. A method of making the population of alphavirus replicon particles of claim 22, comprising:

- A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:
  - (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an

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*env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

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B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences

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encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

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(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

(b) producing the alphavirus particles in the helper cell; and

(c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 22.

25. The method of claim 24, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises nucleic acid sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

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26. A method of making the population of alphavirus replicon particles of claim 23, comprising:

A) (a) providing a first helper cell for producing a first population of infectious, replication defective alphavirus particles, comprising in an alphavirus-permissive cell:

(i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the first population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

(b) producing the alphavirus particles in the helper cell; and

(c) collecting the alphavirus particles from the helper cells;

B) (a) providing a second helper cell for producing a second population of infectious, replication defective alphavirus particle, comprising in an alphavirus-

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permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the second population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells;

C) (a) providing a third helper cell for producing a third population of infectious, replication defective alphavirus particles, comprising in an alphavirus-

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permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;
- (ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and
- (iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the third population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture, and further wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cells; and

D) combining the first population of alphavirus particles produced from the first

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helper cell, the second population of alphavirus particles produced from the second helper cell and the third population of alphavirus particles produced from the third helper cell, thereby producing the population of alphavirus replicon particles of claim 23.

27. The method of claim 26, wherein the alphavirus replicon RNA of at least one of the first helper cell, the second helper cell and the third helper cell comprises nucleic acid sequence encoding at least one alphavirus structural protein and wherein the first helper RNA and the one or more additional helper RNA(s) in the at least one of the first helper cell, the second helper cell and the third helper cell, encodes at least one other alphavirus structural protein not encoded by said replicon RNA.

28. The method of claim 26, wherein only at least one of the first population of alphavirus particles, the second population of alphavirus particles and the third population of alphavirus particles comprises particles wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations.

29. A population of alphavirus replicon particles produced by the method of claim 24.

30. A population of alphavirus replicon particles produced by the method of claim 26.

31. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 21 in a pharmaceutically acceptable carrier.

32. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 22 in a pharmaceutically acceptable carrier.

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33. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 23 in a pharmaceutically acceptable carrier.

34. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 29 in a pharmaceutically acceptable carrier.

35. A method of inducing an immune response to human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 30 in a pharmaceutically acceptable carrier.

36. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 21 in a pharmaceutically acceptable carrier.

37. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 22 in a pharmaceutically acceptable carrier.

38. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 23 in a pharmaceutically acceptable carrier.

39. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 29 in a pharmaceutically acceptable carrier.

40. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the population of claim 30 in a pharmaceutically acceptable carrier.

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41. An alphavirus replicon virosome comprising an alphavirus replicon RNA encapsidated by a lipid bilayer comprising alphavirus glycoproteins, E1 and E2.
42. The virosome of claim 41, wherein the alphavirus glycoproteins are Venezuelan Equine Encephalitis glycoproteins E1 and E2.
43. A method of producing the alphavirus replicon virosome of claim 41, comprising:
- a) combining alphavirus replicon RNA, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and
  - b) gradually removing detergent, whereby alphavirus replicon virosomes are produced.
44. An alphavirus replicon virosome produced from the method of claim 43.
45. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon virosome of claim 41 in a pharmaceutically acceptable carrier.
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46. ~~A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon virosome of claim 41, wherein the virosome comprises alphavirus replicon RNA encoding one or more human immunodeficiency virus immunogens.~~
47. A composition comprising a population of alphavirus replicon virosomes comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus,

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wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon virosome.

48. A composition comprising a population of alphavirus replicon virosomes comprising two or more isolated nucleic acids selected from the group consisting of 1) an isolated nucleic acid encoding an *env* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, 2) an isolated nucleic acid encoding a *gag* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles by the *gag* gene product or the immunogenic fragment thereof and their release from a cell, and 3) an isolated nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation of reverse transcriptase activity in the *pol* gene product or immunogenic fragment thereof, and wherein the nucleic acids are each contained within a separate alphavirus replicon virosome.

49. A method of producing the population of alphavirus replicon virosomes of claim 47, comprising:

A) (a) producing a first population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding an *env* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

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b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

B) (a) producing a second population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding a *gag* gene product or immunogenic fragment thereof, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

C) (a) producing a third population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding a *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced; and

D) combining the first population of alphavirus replicon virosomes, the second population of alphavirus replicon virosomes and the third population of alphavirus replicon virosomes to produce the population of alphavirus replicon virosomes of claim 47.

50. A method of producing the population of alphavirus replicon virosomes of claim 48, comprising:

A) (a) producing a first population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding an *env* gene

product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

B) (a) producing a second population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding a *gag* gene product or immunogenic fragment thereof, wherein the *gag* gene product or immunogenic fragment thereof is modified to inhibit formation of virus-like particles containing the *gag* gene product or the immunogenic fragment thereof and their release from a cell, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced;

C) (a) producing a third population of alphavirus replicon virosomes by combining alphavirus replicon RNA comprising nucleic acid encoding a *pol* gene product or immunogenic fragment thereof, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in inactivation of reverse transcriptase activity in the *pol* gene product or immunogenic fragment thereof, alphavirus glycoproteins E1 and E2, non-cationic lipids and detergent; and

b) gradually removing detergent, whereby alphavirus replicon virosomes are produced; and

D) combining the first population of alphavirus replicon virosomes, the second population of alphavirus replicon virosomes and the third population of alphavirus replicon virosomes to produce the population of alphavirus replicon virosomes of claim 48.

51. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 47, in a pharmaceutically acceptable carrier.

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52. A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 48, in a pharmaceutically acceptable carrier.

53. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 47, in a pharmaceutically acceptable carrier.

54. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 47, in a pharmaceutically acceptable carrier.

55. A composition comprising heparin affinity-purified alphavirus replicon particles, wherein the alphavirus replicon particles comprise at least one structural protein which comprises one or more attenuating mutations.

56. A method of preparing the heparin affinity-purified alphavirus particles of claim 55, comprising:

- a) producing alphavirus replicon particles, wherein the alphavirus replicon particles comprise at least one structural protein which comprises one or more attenuating mutations;
- b) loading the alphavirus replicon particles of step (a) in a heparin affinity chromatography column;
- c) eluting the particles from the column of step (b) with a salt gradient; and
- d) collecting the fraction from the column which contains the heparin affinity-purified alphavirus replicon particles.

57. A composition produced by the method of claim 56.

58. A method of producing a virus replicon particle for use in a vaccine comprising:

- a) producing a plasmid encoding the nucleotide sequence of an alphavirus

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replicon RNA;

- b) producing a plasmid encoding the nucleotide sequence of one or more helper RNAs;
- c) transcribing the plasmids of steps (a) and (b) into RNA *in vitro*;
- d) electroporating the RNA of step (c) into a Vero cell line; and
- e) purifying virus replicon particles from the Vero cell line of step (d) by heparin affinity chromatography.

59. The method of claim 58, wherein the virus replicon particles are produced in large-scale.

60. Virus replicon particles produced by the method of claim 59.

61. An isolated nucleic acid encoding a *pol* gene product or immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof.

62. A composition comprising the nucleic acid of claim 61.

63. A vector comprising the nucleic acid of claim 61.

64. A cell comprising the vector of claim 63.

65. An alphavirus replicon particle comprising the nucleic acid of claim 61.

66. A method of making the alphavirus replicon particle of claim 65, comprising

- a) providing a helper cell for producing an infectious, defective alphavirus particle, comprising in an alphavirus-permissive cell:

- (i) an alphavirus replicon RNA, wherein the replicon RNA

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comprises an alphavirus packaging signal and a nucleic acid encoding a *pol* gene product or an immunogenic fragment thereof of a human immunodeficiency virus, wherein the *pol* gene product or immunogenic fragment thereof comprises a modification resulting in deletion or inactivation of protease, integrase, RNase H and reverse transcriptase functions in the *pol* gene product or immunogenic fragment thereof, and wherein the replicon RNA lacks sequences encoding alphavirus structural proteins;

(ii) a first helper RNA separate from said replicon RNA, said first helper RNA encoding at least one alphavirus structural protein and furthermore not encoding at least one other alphavirus structural protein; and

(iii) one or more additional helper RNA(s) separate from said replicon RNA and separate from said first helper RNA, said additional helper RNA(s) encoding at least one other alphavirus structural protein not encoded by said first helper RNA;

and with at least one of said helper RNAs lacking an alphavirus packaging signal;

wherein the combined expression of the alphavirus replicon RNA and the helper RNAs produces an assembled alphavirus particle which is able to infect a cell, and is unable to complete viral replication, and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture;

- (b) producing the alphavirus particles in the helper cell; and
- (c) collecting the alphavirus particles from the helper cell.

67. The method of claim 66, wherein at least one of said replicon RNA, said first helper RNA, and said one or more additional helper RNA(s) comprises one or more attenuating mutations.

68. An alphavirus replicon particle produced according to the method of claim 66.

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69. An alphavirus replicon particle produced according to the method of claim 67.

70. A method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 62 in a pharmaceutically acceptable carrier.

71. A method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon particle of claim 65 in a pharmaceutically acceptable carrier.

72. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the composition of claim 62 in a pharmaceutically acceptable carrier.

73. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of the alphavirus replicon particle of claim 65 in a pharmaceutically acceptable carrier.

74. A method of inducing an immune response in a subject, comprising administering to the subject an immunogenic amount of a composition comprising the alphavirus replicon particles of claim 65 in a pharmaceutically acceptable carrier.

75. A method of treating or preventing infection by human immunodeficiency virus in a subject, comprising administering to the subject an immunogenic amount of a composition comprising the alphavirus replicon particles of claim 65 in a pharmaceutically acceptable carrier.

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